Mechanisms of transcriptional regulation by Cyc8p-Tup1p corepressor/coactivator in *Saccharomyces cerevisiae*

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Transcriptional regulation is crucial in gene regulation for various cellular and organismal events. Especially, transcription initiation control is the most important step for gene expression. Transcriptional activation and repression in eukaryotes are regulated by activators and repressors, which bind to promoter regions of genes to recruit non-DNA-binding transcription factors, coactivators and corepressors. The budding yeast *Saccharomyces cerevisiae* Cyc8p-Tup1p protein complex is well-studied as a corepressor. The Cyc8p-Tup1p complex, which consists of one Cyc8p and four Tup1p subunits, is recruited to target gene promoters by associating with DNA-binding repressors and represses transcription of the genes through inhibiting function of transcriptional machinery and establishing repressive chromatin structure by interaction with low-acetylated histones H3 and H4. Cyc8p-Tup1p complex is also reported to function as a coactivator, and is therefore categorized as a coregulator. In this thesis study, I investigated transcriptional regulatory mechanisms of Cyc8p-Tup1p corepressor/coactivator.

Tup1p has three functional domains: the N-terminal tetramerization and Cyc8p-interaction domain (1-72 amino acids), the middle histone-binding domain (73-328 amino acids), and the C-terminal repressor-binding WD-repeat domain (329-713 amino acids). A crystal structure of the N-terminal 92 amino acids of Tup1p was determined and revealed that it forms a 4-helix bundle structure. To identify amino acid residues that are important for transcriptional repression in the Tup1p N-terminal domain, site-directed mutagenesis based on its three-dimensional structure was performed to analyze repression function of the resultant mutants. Negatively charged residues on the surface of the tetramer of Tup1p were not required for transcriptional repression. Three

consecutive glutamates at positions 74-76 and nonpolar residues inside the 4-helix bundle were important for transcriptional repression, considering association of histones and stabilization of tetrameric structure, respectively.

The middle domain of Tup1p is redefined as 93-281 amino acids region based on structural information of the N-terminal and C-terminal domains. To investigate whether this middle domain is important for transcriptional repression, *tup1*Δ93-281 mutant was constructed. The *tup1*Δ93-281 mutant exhibited flocculation phenotype as did *TUP1*-deleted strain, but had a normal mating efficiency comparable to the wild type. DNA microarray analysis showed that the middle domain of Tup1p is not necessarily required for transcriptional repression of Tup1p target genes. Glucose-repressible genes that are regulated by Mig1p repressor were not repressed in the *tup1*Δ93-281 mutant, whereas mating-type-specific genes (regulated by Matα2p) were repressed. Tup1Δ93-281p was recruited to both promoter regions of glucose-repressible *SUC2* and **a**-cell-specific *STE2* gene. These results indicate that the necessity of middle domain of Tup1p for repression is classified by particular pathway-specific repressors. These suggest that Tup1p might bind to histones through a region excluding the middle domain, or could repress transcription of genes without binding to histones.

The *CYC8*- and *TUP1*-deleted strains show the same pleiotropic phenotypes, however, *CYC8*-deleted strain grows extremely slower than wild-type and *TUP1*-deleted strains. To understand the cause of the growth defect of Δ*cyc8* cells, multicopy suppressor genes for *CYC8* deletion mutation were isolated: tryptophan transporter genes *TAT1* and *TAT2*. Transcriptional levels of *TAT1* and *TAT2* were reduced in both Δ*cyc8* and Δ*tup1* cells. Addition of tryptophan to cultures partially improved growth of Δ*cyc8* cells. These showed that Cyc8p and Tup1p activate transcription of the *TAT* genes, and suggested that decreased tryptophan uptake activity followed by intracellular tryptophan deficiency might be a cause of growth defect by *CYC8* deletion. *CYC8* and *TUP1* were also required for transcription of amino acid transporter genes that are regulated by DNA-binding activators Stp1p/Stp2p. Tup1p bound to the *TAT* gene promoters dependent on Stp1p/Stp2p. These data indicate that Cyc8p-Tup1p plays a role as a transcriptional coactivator for *TAT* genes, probably also for the other amino acid

transporter genes, via Stp1p/Stp2p activators.

In this thesis study, I revealed molecular functions of Cyc8p-Tup1p complex as a transcriptional coregulator based on structural information. Further, I discussed that mechanisms of transcriptional regulation of genes that are regulated by a particular activator or repressor are not unique and therefore a general model that is constructed combining the knowledge accumulated from various investigations could not be necessarily applied to regulation of all target genes. These information would be helpful for researchers who study transcriptional regulation in higher eukaryotes.